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UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Thomas Rausch

Serial No.: 09/762,782

Group Art Unit: 1638

Filed: March 30, 2001

Examiner: R.Kallis

For: TRANSGENIC PLANTS AND CELLS COMPRISING A REDUCED EXPRESSION OF
PLANT INVERTASE INHIBITORSAssistant Commissioner for Patents
Washington D.C. 20231DECLARATION OF THOMAS RAUSCH UNDER 37 C.F.R. §1.132

Sir:

I, Thomas Rausch, having residence at Im Neuenheimer Feld 360, D-69120-
Heidelberg, Germany, hereby declare that

1. I am inventor of the above-identified application and am familiar with the subject application.
2. Total RNA was isolated from *B. napus* siliques (6 days-after-pollination), and mRNA was purified by oligo-dT cellulose chromatography. A cDNA library was prepared in ZAP-Express EcoRI/XhoI (Stratagene). The library was screened with a biotinylated probe corresponding to the *A. thaliana* *Bn-inhh1* homolog with the accession number AA712621 (this EST clone showing significant homology to the tobacco *Nt-inh1* cDNA; Greiner et al., 1998). The obtained *Bn-inhh1*-full length clone was sequenced in both directions (for cDNA sequence see below). The *Bn-inhh1* cDNA was cloned in antisense orientation behind the 35S promoter into the pBinar vector for *A. tumefaciens*-mediated transformation of *B. napus* (Drakkar). Following this procedure, a transgenic rape plant was generated, which showed an increased seed weight and seed storage oil content.

a) Reserve material content of seeds of a transgenic rape plant transformed with a rape invertase inhibitor sequence (Bn-Inhh1) in comparison to wild-type plants:

Parameter	Rape (type Drakkar, WT)	Antisense-transformants (type Drakkar)		
		Line 11	Line 13	Line 24
Average weight of seed	3.8 ± 0.3 mg	4.1 ± 0.4 mg	4.2 ± 0.3 mg	4.4 ± 0.4 mg
% w/w oil content	43 ± 3 %	55 ± 7 %	56 ± 5 %	53 ± 4 %

b) Protein sequence of the rape Invertase Inhibitor isoform (Bn-inhh1) in comparison to the protein sequence of the tobacco Invertase Inhibitor (Nt-Inh1; Greiner et al., 1998):

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      *           20           *           40           *
Ntinhl : -----MKNLIFLTMTFLTILLQTNANNLVETTCKNTFPNYQLCLKTLLSD : 43
Bninhh1 : -----MCKMVVVKMDMMVTVMVSEASMIETCKETPDENLCVSLDSD : 43

      60           *           80           *           100
Ntinhl : KRSATGDITTLALIMVDAIKAKANQAAVTISKLRHSNPP--AAWKGPLKN : 91
Bninhh1 : PRGSSADTSGLALILVDKIYKGLATKTLKEINSLYKGRP----ELKQALDE : 89

      *           120           *           140           *
Ntinhl : CAFSYKVIITASLPEAIEALTKGDPKFAEDGMVGSQDAQECSEYFKGSK : 141
Bninhh1 : CSRRYKTIILKADVPEAIEATSKGVPKFGEDGVIDAGVEAAVCEGEFKG-K : 138

      160           *           180
Ntinhl : -----SPFSALNIAVHETSDVGRAIVRNIL~ : 166
Bninhh1 : -----SPLTSLTKSMQKVSINVTRAIVRMIL~ : 163

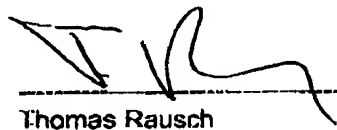
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c) Nucleic acid sequence coding for Bn-inhh1

ATGAAGAAGATGGTGGTGGTGAAGATGATCATGATGGTAACAGTGATGGTGAGTGAAGCGAGTATGAT
AGAGCAGACATGCAAAGAGACACCAGATTTCAATCTCTGCGTCTCTCTCTCGACTCCGACCCACGTG
GCTCCTCTGCCGACACCTCTGGCCTCGCTCTTATCCTCGTCGATAAAATCAAGGGGTTGGCGACGAAG
ACCTTGAAAGAAATCAACAGTCTATATAAAAAGAGACCGGAAGTGAACAGGCTTTAGACGAATGTAG
TCGAAGATACAAAACGATCTTAAAGGCTGATGTTCCCGAAGCCATTGAAGCTATATCTAAAGGCGTTC
CGAAGTTCGGTGAAGACGGTGTGATCGATGCTGGTGTGGAAGCTGCTGTATGTGAAGGAGAAATTTAA
GGGAAATCTCCGTTGACTAGTTTGACCAAATCAATGCAAAAAGTCTCTAATGTGACTAGAGCCATTGT
GAGAATGTTGCTTTGATTTTATCTTTTATGTTTCGTTCATACAAGCATGTGGTATGGGTGTTTATGGG
AT

3. I further declare that all statements made herein are made of my own knowledge and are true except for those statements made on information and belief, which are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willfull false statements may jeopardize the validity of this declaration of this application and any United States patent issuing therefrom.

Feb 9th 2003
Date


Thomas Rausch